# Chemical states of the N-terminal "lid" of MDM2 regulate p53 binding Simulations reveal complexities of modulation

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# Chemical states of the N-terminal "lid" of MDM2 regulate p53 binding

## Simulations reveal complexities of modulation

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Phosphorylation of S17 in the N-terminal "lid" of MDM2 (residues 1–24) is proposed to regulate the binding of p53. The lid is composed of an intrinsically disordered peptide motif that is not resolved in the crystal structure of the MDM2 N-terminal domain. Molecular dynamics simulations of MDM2 provide novel insights into how the lid undergoes complex dynamics depending on its phosphorylation state that have not been revealed by NMR analyses. The difference in charges between the phosphate and the phosphomimetic 'Asp' and the change in shape from tetrahedral to planar are manifested in differences in strengths and durations of interactions that appear to modulate access of the binding site to ligands and peptides differentially. These findings unveil the complexities that underlie protein-protein interactions and reconcile some differences between the biochemical and NMR data suggesting that lid mutation or deletion can change the specific activity of MDM2 and provide concepts for future approaches to evaluate the effects of S17 modification on p53 binding.



### Introduction

The ubiquitin ligase MDM2 negatively regulates the tumor suppressor protein p53 in normal cells by targeting it for degradation.[1,2] In stressed cells the MDM2-p53 equilibrium shifts towards dissociation as a result of post-translational modifications (PTM), e.g., phosphorylations. This leads to the accumulation of p53 that triggers cell repair or apoptotic pathways.[1,2] Multiple phosphorylation sites have been identified on either partner of the p53-MDM2 complex including the transactivation (TA) domain of p53 and the p53 binding N-terminal domain of MDM2. These regions have been well characterized and the role of phosphorylation in cellular regulation has been unambiguously described in references 3–7. However, the exact mechanisms underlying this regulation are still being unraveled. The first 24 amino acids of the p53 binding N-terminal domain of MDM2, well conserved in mammals, are thought to form a disordered segment that occludes the p53 binding site on MDM2 in a pseudo-substrate, lid-like fashion; details however remain enigmatic.[8] Based on biochemical data[9] and NMR studies[8] it was first hypothesized that upon stress, S17 that lies in this lid region (**Fig. 1 and Sup. Fig. 1**), gets phosphorylated by DNA-PK (DNA-dependent protein kinase) and interacts with high affinity with the region surrounding the MDM2 cleft and that this would inhibit the p53-MDM2 interaction. NMR studies have revealed that the apo-form of MDM2 is in a distinct conformation compared to ligand bound MDM2 and revealed that the lid can exist in several interconverting dynamic conformations in the ligand-free state.[10] NMR also suggested a set of residues in MDM2 that appear to respond to the presence of the negative charge at position 17 (in the form of a phosphomimetic S17D); of these, H96 and K98 are the residues likely to be involved in charge-charge interactions with D17, which results in the occlusion of the pocket of MDM2 from p53.[8,11] However, despite the fact that NMR can be used to form predictions on how the phospho-mimetic lid inhibits the formation of the MDM2-p53 complex, associated experimental data in vitro or in vivo has been limited.

Enzymological studies[12] have since shown that the phosphomimetic S17D can promote the binding of p53 to MDM2, and can increase the ubiquitination of p53 in vitro. These enzymological studies contrast to the predictions based on NMR and was rationalized through modeling which suggested that D17 forms a salt bridge (SB) with R97 and K98, both of which are located on the surface of MDM2 but away from the binding pocket. This could lock the lid in a conformation that keeps the binding pocket 'open'. Consistent with this, phospho-mimetic lid mutation also increases the thermostability of the N-terminal domain of MDM2 in the presence of Nutlin or p53-mimetic peptides.[13] This is in contrast to the NMR derived hypothesis that the

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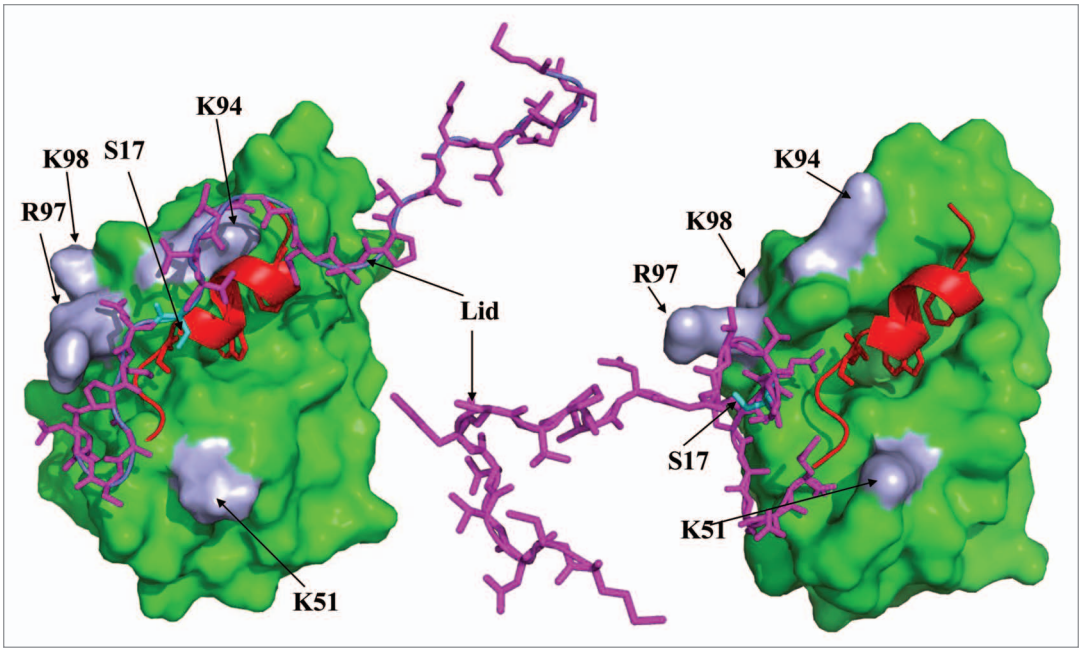


**Figure 1.** Two different structures from the NMR ensemble showing the closed (left) and open (right) conformation of MDM2's lid; placing the p53 peptide into MDM2's binding cleft shows that steric occlusion is the reason why the 'closed' conformation cannot accommodate p53 whereas the 'open' conformation can; MDM2's cleft (green) & lid (magenta), p53 (red); few important residues are highlighted with different colors.

**Table 1.** List of notation used for the trajectories

| | Open | Closed | Open (helix) | Closed (helix) |
|---|---|---|---|---|
| WT-MDM2 | WTo | WTc | WTohx | WTchx |
| S17-phosphorylated | S17po | S17pc1, S17pc2 | S17pohx | S17pchx |
| S17D mutant | S17Do | S17Dc | S17Dohx | S17Dchx |
| TM | Tmc | Tmo | x | x |

First row indicates lid conformations, including the cases where 21–24 modeled as an α-helix. First column refers to the WT and mutant systems. TM is the triple mutant 'S17D, R97S, K98P'. Trajectories after swapping of the side chains (S17p/D17), termed as S17p2D and D2S17p (not listed) which has been discussed in the text. See movies in SI.

negative charge on the lid (either through phosphomimetic or through phosphorylation) would lock the lid on top of the active site and prevent p53 from binding. We still do not know whether in vivo the phospho-mimetic MDM2 lid is more or less active towards p53.

The function of the lid of MDM2 is thus being tackled by a combination of structural biology, biophysics, NMR, enzymology and cell biological analyses. The lid function is not yet resolved and for example, it is not known how phosphorylation would actually change MDM2 conformation since the NMR and enzymological studies assumed that the phosphomimetic lid mutation represents what occurs when S17 is phosphorylated (pS17). Contrasting observations have also been obtained from NMR experiments; for example one study of the N-terminal domain of MDM2 suggested striking changes in MDM2 conformation upon ligand binding,[14] whilst another NMR study on the same MDM2 domain indicated that no conformational change occurred upon ligand binding.[11] Molecular dynamics (MD) simulations based on the crystal structure of MDM2 offer the potential to provide novel insight into how the lid undergoes complex dynamics depending on its phosphorylation state. The difference between an Asp (or Glu) mimicking phosphorylation, and an actual phosphate group is that the former is planar and carries a net charge of -1 while the latter is tetrahedral and carries a net charge of -2 (normally). This would immediately suggest differences in the lifetimes and strengths of interactions with charged residues. Indeed, recent simulation studies combined with experiments have shown that these two could give rise to significant differences.[15,16] In order to probe these differences in the MDM2-p53 system, we carried out detailed molecular dynamics simulation studies and provide hypotheses than can lead to novel experimental approaches to study MDM2 regulation. We have simulated the wild type (WT), S17D and the pS17 states of p53 binding domain of MDM2 (residues 1–119) with the lid in 'open' and 'closed' conformations (see **Fig. 1**) which have been listed in **Table 1** with their notations used in the following text.

## Results and Discussions

**WT.** The lid region [MCNTNMSVPT$_{10}$ DGAVTTSQIP$_{20}$ ASEQ$_{24}$] is Ser-Thr rich and has two anionic residues (D11 and E23). The p53 binding cleft of MDM2 is largely positively charged with several cationic residues in its vicinity (K51, K64, R65, K70, K94, R97, K98; see **Sup. Fig. 2**) and it is clear that this region can attract the negatively charged lid, albeit some parts of it. Aromatic residues F19/W23 of the TA domain of p53 (See **Sup. Fig. 3**) have been established as the main drivers of the high

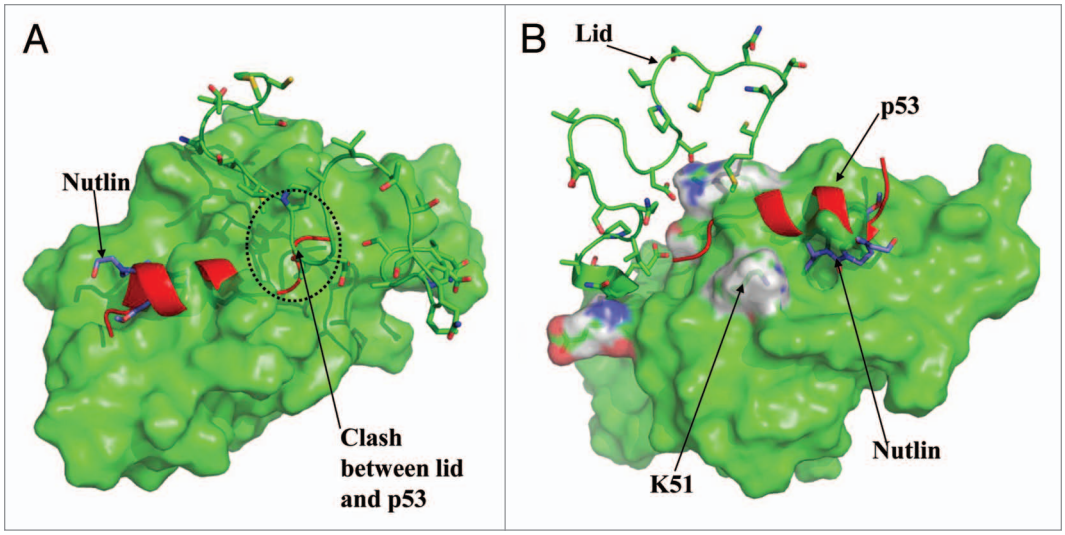


**Figure 2.** (A) Snapshots from the WTc trajectory showing a lid conformation that clashes with a p53 peptide (red) but can allow the small molecule inhibitor 'nutlin' (purple) to bind (built by superimposing MDM2-p53/nutnil complexes from PDB: 1YCR & 1RV1); (B) Snapshot from the WTchx trajectory showing how the binding of both peptide and small molecule nutlin is possible.

affinity interaction between this domain of p53 and the hydrophobic p53 binding cleft of MDM2,[3,17] and it is noteworthy that the lid lacks aromatic side chains. Thus the lack of a suitable anchor in the lid results in its high mobility even when it covers the pocket, as is clear from the simulations (see **Movie M1a-b**). In one particular region of its conformational space, the lid is held to the cleft by K94-D11 (stable ~5 ns) (**Sup. Fig. 4**) and R97-E23 salt bridges (stable ~14 ns) and hydrophobic interactions between I19 and the cluster of L54, H96 and Y100 of the MDM2 cleft; the lid lies over the binding pocket, which remains closed. Interestingly, simulation studies had suggested that this cluster is responsible for mediating the binding of p53.[4] In yet another region of the conformational space of the lid, the K94-D11 interaction is replaced as the K94 side chain coordinates the backbone and side-chains of other residues such as N3, N5 etc., and the D11 is stabilized by Y67 and Q72 for another 1.5 ns, after which it gets exposed to solvent. The breaking of the K94-D11 interaction results in the lid moving away from the surface (see **Movie M1b**) leaving the p53 binding site accessible to the solvent. This is accompanied by the rearrangement of a few residues around the binding pocket (L54, M62, Y67, H96 and Y100) that cover the opening of the pocket (**Sup. Fig. 5**) and appear to occlude the hydrophobic pocket from water molecules. The R97-E23 interaction seems to enable the association of the A21-Q24 region against the cleft that packs Y100 (together with H96, M62 and Y67) and breaks at ~14 ns after which the lid remains exposed to solvent for another ~2 ns, when it folds on itself. At this stage the binding cavity appears to be sufficiently exposed for small molecules such as 'nutlin'[18] to bind (**Fig. 2**) although the gatekeeper Y100,[4] remains in the closed conformation; nevertheless the binding site is open enough for initial encounter complexes to form.[19,20] This now provides a molecular picture behind the experimental observations[11,13] whereby the presence of the lid can occlude MDM2 from binding p53 and yet allow small molecules (and short peptides as is apparent from our simulations) such as nutlin to bind.

We further model the region 21–24 at the C-terminal end of the lid as α-helical (based on experimental observations[10]), and find that it remains stable for 10 ns. Three salt bridges stabilize the lid: D11-K94 (during 0–1 ns), E23-R97 (during 0–2 ns) and D11-R97 (during 7.5–10 ns) (**Fig. 3 and see Movie M1c-d**), including a very transient (<0.5 ns) E25-K51 interaction. In the presence of the helical conformation, D11 initially interacts with K94. However, the dynamics of the lid are associated with rapid rearrangements leading to the collapse of the lid onto the surface. By ~1.2 ns, the E23-R97 salt bridge is broken and Y100 begins to flip out of its closed state. Finally the helix undergoes a shift as Y100 makes stabilizing interactions with D23, while R97 is stabilized by D11. E25 and K51 are both solvent exposed. The flipping out of Y100 leads to an active site that is more open than in the non-helical lid considered above. **Figure 3B** shows how this conformation can easily bind both p53 and nutlin.

**pS17.** Phosphorylation of S17 in WTc was investigated by running two separate simulations: S17pc1 and S17pc2. In S17pc1, initially the phosphate group is hydrated and is stabilized by hydrogen bonds (HB) to T16. Although the simulation started with the lid in a 'closed' conformation, there is an initial transient 'opening' that leaves the active site largely accessible, but by ~4 ns the active site is completely occluded by the lid and by ~8.5 ns, K51 moves towards the phosphate group at S17 and forms a salt bridge that remains stable until the end of 20 ns (**Fig. 4 and Movie M2b-c**). D11 forms a salt bridge with K94 while R97 forms a salt bridge with E114 which lies in the C-terminal tail. E23 and E25 remain hydrated. In the other 20 ns simulation (S17pc2), the lid undergoes rapid rearrangements by 7 ns and now the phosphate at S17 makes a salt bridge with K94 (**Fig. 4**). This is accompanied by interactions with the side chains of H96 and Y100, with the latter undergoing a transition to

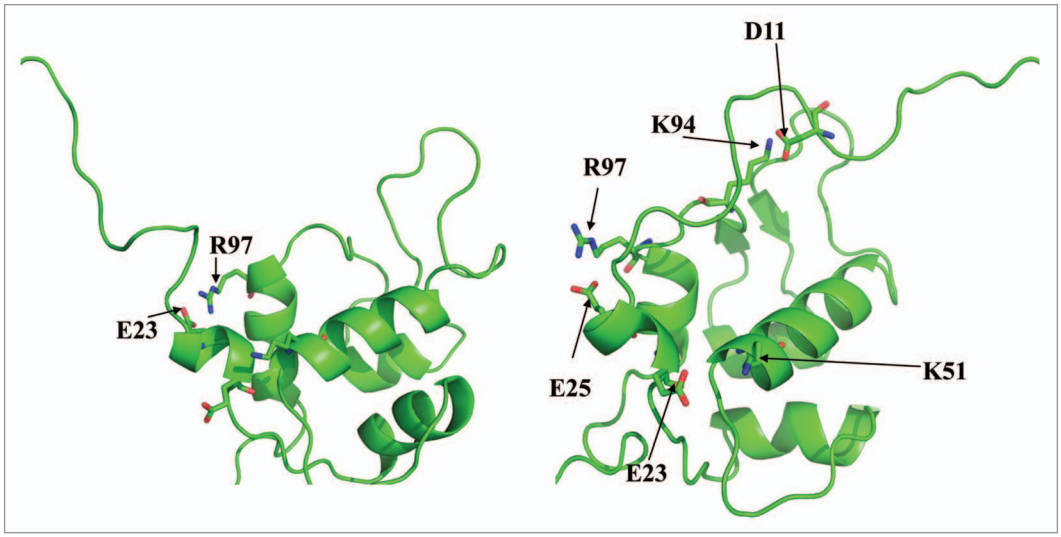


**Figure 3.** Representative snapshots from WTohx (left) and WTchx (right) trajectories showing stabilizing interactions.

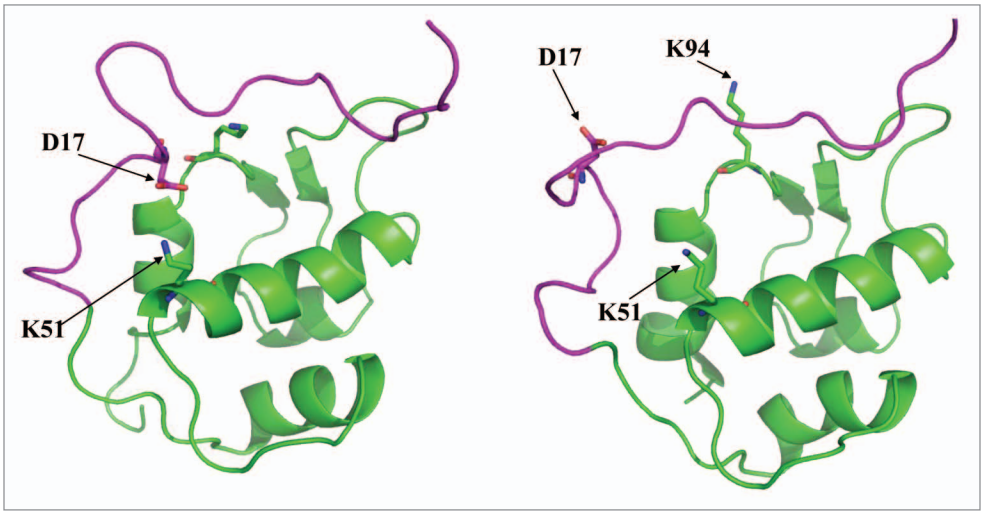


**Figure 4.** Snapshots from S17pc1 (left) and S17pc2 (right) trajectories show the salt bridge formation of pS17 with K51 and K94.

the "open state"[4] by 9 ns. At this point, the active site remains very much open for binding to nutlin and possibly short helical peptides (but not p53, see **Sup. Figs. 6 and 7**); by 17 ns, R97 also makes an interaction with the phosphate. This latter observation seems to be in accord with the experimental findings of Worrell et al.[13] To get an estimate of the strength of the interaction between the lid and the cleft upon phosphorylation (at S17), the -2e charge on the phosphate is switched off, and we find a 110 kcal/mol destabilization in the electrostatic energy and a further loss of ~286 kcal/mol of solvation energy, thus highlighting the strong electrostatic interactions between the phosphate and the MDM2 surface. Our results now build upon experimental observations,[8,11] demonstrating that the lid is very flexible and not stabilized in any specific location on the cleft. This flexibility is necessarily attenuated upon phosphorylation as the lid will now preferentially be stabilized at the cationically dense regions of the cleft (**Sup. Fig. 2**) (at least two regions of the MDM2 surface are apparent from our simulations, a feature that has also been reported earlier[20]).

WTc showed a transition of the lid from a closed state to an open state during the simulation, suggesting a very weak association; however the transition from open to closed state was not observed for either WTc and WTo (see **Movie M1a-d**) the timescale of this equilibrium exists in the millisecond regime.[11] In contrast, for S17pc1&2, the lid opens during the first 1–2 ns but closes between 2–4 ns as the phosphate group localizes at K51 or K94 (**Fig. 4 and Movie M2b-c**); this suggests that the phosphorylated lid clearly has a higher affinity for the cleft. When S17po was simulated, the phosphate forms salt bridges with R97 or K98 (**Sup. Fig. 8**) leaving the binding pocket open to a ligand (**Movie M2a**). In summary, simulations suggest that when the lid is phosphorylated, it can adopt distinct conformations that are driven by salt bridging of the phosphate with (1) K51 (binding site is occluded), (2) K94 (binding site is occluded) and

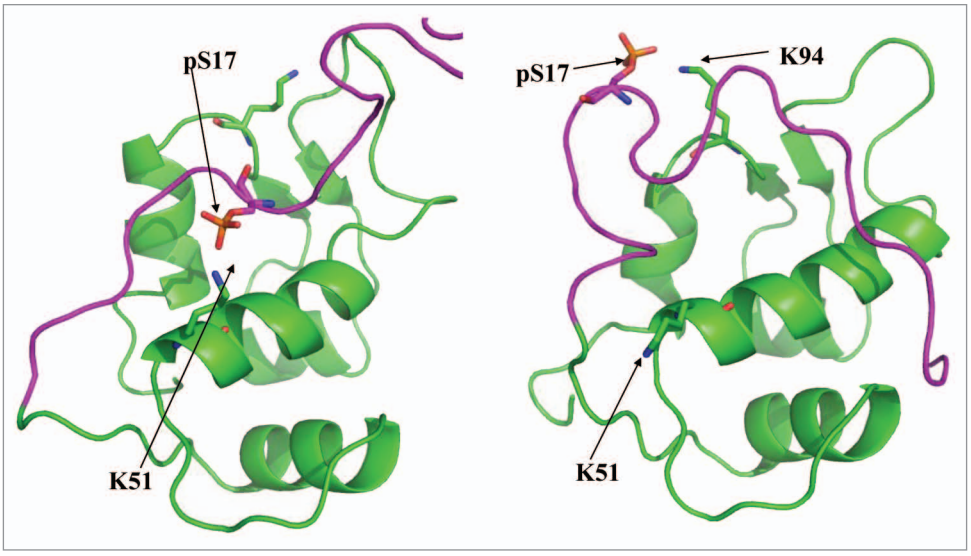


**Figure 5.** Snapshots from the beginning (left) and end (right) of 10 ns of S17Dc, showing the instability of D17-K51 salt bridge leading to changes in lid conformation.

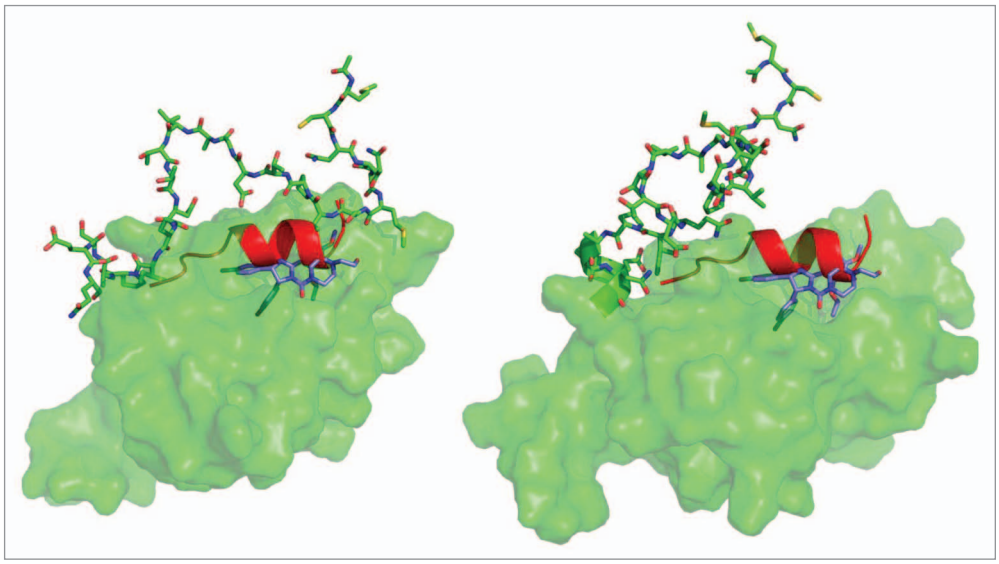

**Figure 6.** Two different snapshots from S17Dc show that conformation of the lid leaves the binding pocket accessible to both p53 (red) and 'nutlin' (purple) (superimposed MDM2-p53/nulin complexes from PDB).

(3) R97/K98 (binding site is relatively exposed). The relative population of these two states (closed and open) in the phosphorylated structure would determine the effect of phosphorylation on the equilibrium of the MDM2-p53 complex formation. If the 21–24 region is helical, the phosphate is stabilized by K51 and the lid completely covers the binding site throughout the simulation (S17pchx, see **Sup. Fig. 9 and Movie M2e**). Additionally there may be as yet undiscovered protein partners that may regulate this; or as Worrall et al.[12] have suggested, there may be allosteric effects of the interactions between the lid and the RING domain of MDM2 that have effects on ligand interactions.

**S17D.** Simulation of S17D started in the closed state (S17Dc) shows transient interactions of D17 with K51, H96 and K94 during the first 4 ns but eventually the system stabilizes with the D17 located in the vicinity of K97/R98 (**Fig. 5**), as observed during the last 2 ns of the total 10 ns trajectory. The binding site is open and can accommodate small molecules such as nutlin and p53 (**Fig. 6 and Movie M3b**). When the 21–24 region is modeled as helical (**Sup. Fig. 10**), simulations (S17Dchx) show that the lid is stabilized in an open state with a D17-K51 and E25-R97 salt bridges (lasts most of the simulation) and the active site can accommodate nutlin and p53 peptides. This brings together the observation that the 21–24 region may be helical as determined by NMR and that the S17D mutant binds peptides.[12,13] Simulation of S17D from the open state (S17Do, **Movie M3a**) shows that the loop can be stabilized either with R97 (4–8 ns)

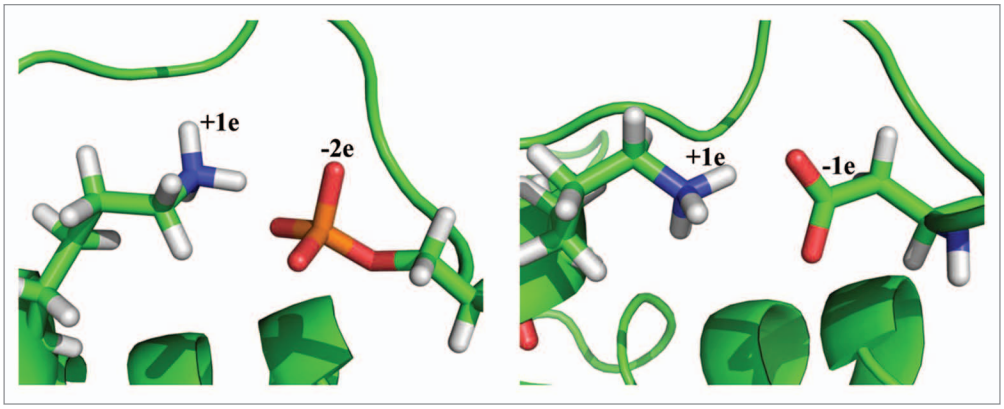


**Figure 7.** Representative snapshots showing the salt bridges between K51 and pS17/D17; the differences in interactions due to the tetrahedral/planar geometry of functional groups is clear.

or with K51 (~10 ns). Again in the open state with 21–24 helical, D17 is stabilized either by R97 (~1.5 ns) or else remains hydrated with the binding site remaining open (see **Movie M3c**). Indeed, in the triple mutant TM (R97S, K98P, S17D)[12] the above interactions are absent and the lid tends to form a E25-K51 salt bridge as well as a less stable interaction between D17 and K51; in 'TMo' the lid moves away from the binding pocket leaving it completely open (see **Sup. Fig. 11**) and in 'TMc' also its shows large flexibility (see **Movie M4a-b**). To get an estimate of the strength of the interaction of the phosphomimetic with MDM2, if the charge of -1e on D17 is switched off, the electrostatic energy is destabilized by ~80 kcal/mol and the solvation energy by ~30 kcal/mol, which is much smaller in magnitude compared to that observed for the phosphate group.

MD simulations were carried out to investigate the dynamics of regulation of the p53-MDM2 interactions. This interaction is known to be under the regulation of phophorylations;[2] however mechanistic details are incomplete although some insights have been obtained from modeling inspired mutagenesis studies.[12,21,22] The lid domain of MDM2 has been implicated in this regulation, through the phosphorylation of S17. Earlier work[8] had hypothesized that under normal, unstressed conditions, the lid interacts weakly with the surface of MDM2 and is easily displaced by p53 which is then bound by MDM2 and removed via the ubiquitination-proteasomal degradation pathway.[1,2] Upon stress, the lid is phosphorylated at S17 and the newly acquired negatively charged phosphate group on the lid interacts strongly with residues on the MDM2 surface that lie in the vicinity of the p53 binding groove and occludes the groove from p53, which is then stabilized to carry out its function. This model was complicated by the observation that the lid can adopt conformations whereby it can occlude the groove from p53 and yet allow small molecules such as nutlin to bind.[11] More recently, the work of Worrell et al. have added a new layer of complexity to this picture by showing that upon phosphorylation (using a phosphomimetic) the lid can allow the binding of p53 and of nutlin.[12,13] This at first glance appears to be in contrast to the biological picture where phosphorylation of the lid prevents p53 binding. However we now show, using molecular dynamics simulations, that this apparent difference is resolved once we include the dynamical differences that result from phosphorylation and those that result from using a phosphomimetic. Our study was inspired by earlier successes in reproducing and successfully predicting various features of the p53-MDM2 interaction,[4,19] and recent demonstrations that a phosphomimetic may display dynamical features that can be quite different from those displayed by a phosphate group.[16]

Simulations show that in the WT, the lid indeed has a weak affinity for the p53-binding cleft on the surface of MDM2, as postulated from the experiments. However the lid can also dynamically span several conformational sub-states that can interconvert between open, semi open and closed states (however on the timescales of the simulations we only see the closed to open transition), in agreement with NMR observations.[8,11] Simulations show that associated with lid opening is rearrangements of side chains such as Y67, H96 etc., that can occlude the binding pocket; these sidechains can also undergo conformational changes in the presence of unbound (but associated through long range interactions) p53, which then enables p53 to bind.[19] In a subsequent study,[20] it was demonstrated that the "approach" of p53 and MDM2 is governed by a funnel shaped landscape that is strongly electrostatic in character (although at close range the van der Waals interactions dominate), originating largely in cationic residues surrounding the pocket of MDM2 (e.g., K51, K94, K98, etc.). While the residues K51 and K94 do not take part in interacting with p53 in its bound state, nevertheless they seem to play a significant role in attracting and orienting p53 for appropriate binding. This suggests that if the lid has to prevent complex formation with p53, it could do so by attenuating the effects of these positive charges. We find in our current study that indeed, when S17 is phosphorylated, K51 and K94 interact strongly with the negatively charged phosphate. These residues are well conserved across the different species (**Sup. Fig. 12**). This further suggests that the unit charge difference between the phosphomimetic (S17D) and the phosphate group (pS17) will lead to a greater degree of attenuation by the latter. In addition, the tetrahedral geometry (**Fig. 7**) of the phosphate group is associated with a larger probability of maintaining salt bridge interactions. Indeed in our simulations,

the S17D mutant shows weaker associations of the lid compared to those of S17p and as expected has a somewhat higher stability compared to WT. Due to the weaker association of S17D with K51 or K94, the lid appears to have a higher tendency to move towards the larger positive density presented by K97/R98. These result in a population shift towards the S17Do state characterized by salt bridges between D17 and K97/R98, which is in agreement with the observations of Worrell et al.[12]

It is clear from the simulations that the interactions of pS17 are much more stable compared to those of D17. To further test this, we took representative snapshots from the simulations of pS17 and S17D (after the salt bridges with K51 had been stably formed) and carried out mutations whereby the pS17 was mutated to D17 and the D17 was mutated to pS17 (named as S17p2D and D2S17p respectively) and then continued the simulations. As expected, for the pS17→D17, the interaction with K51 is lost within a nanosecond, while for the D17→pS17 mutation, the salt bridge is maintained for 10 ns (See **Movie M5a-b**).

In effect, MD simulations show that the conformational ensembles that define the lid dynamics differ depending on the chemical state of the lid (S17D or phosphorylated S17). However, reconciliation of the contrasting observations that the S17D mutation appears to occlude p53 from binding (as observed in NMR), or can enable p53 to bind (enzymology) is more difficult. While simulations show that the chemically modified flexible lid can access regions of the conformational space that satisfies both observations (allow small molecules and peptides to bind and prevent longer peptides from binding), they also show that the S17D mutation does not appear to directly access conformations that a phosphorylated S17 does. This once again is a demonstration that comparison of the two chemical states must be carried out with caution especially given the complexities that underlie protein-protein interactions. It is possible, as Worrell et al. (REF) point out, that the differences in the contrasting observations between NMR and enzymology may possibly arise from the fact that enzymology was carried out with full length MDM2, while the NMR data is derived from the Nterminal domain of MDM2. Models of the full length MDM2 are keenly awaited so that the mechanisms underlying allostery may be explored.

In summary, we show for the first time, using MD simulations, how a flexible N-terminus "lid" region of MDM2 can regulate the p53-MDM2 interactions and how some of the differences between various experimental observations can now be reconciled at the molecular level. These differences arise from the differing behavior of the flexible "lid" region depending on its chemical state when it is phosphorylated or when phosphorylation is being mimicked by using a phosphomimetic mutant. It also demonstrates for the first time the complex behavior of these systems and how subtle interactions defined by local differences in charge and geometry can modulate ligand binding/protein-protein interactions. Further mutational studies will undoubtedly throw up additional complexities.

## Methods

MD simulations were carried out on the p53 binding domain of MDM2 (residues 1–119) in its wild type (WT), S17D and the pS17 states with the lid in 'open' and 'closed' conformations (**Fig. 1**). These states were modeled using the structures taken from the NMR derived structural ensemble of 'apo' MDM2 (RCSB code 1Z1M).[10] The NMR data also hinted at the presence of a transient α-helix[10,11] across residues 21–24, and this was modeled too. Standard protocols[4,19] using the CHARMM22 force field[23] were applied to carry out MD simulations at 300 K in explicit water for 10 ns (listed in **Table 1**) with a few simulations extended to 20 ns. This resulted in a total simulation time of ~200 ns. Details of the MD protocols have been provided in the supplementary information (SI).



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### Note

Supplemental materials can be found at:
www.landesbioscience.com/journals/cc/article/14345/
Movies can be found at:
http://web.bii.a-star.edu.sg/bmad/MDM2lid-2010/

### References

1. Brown CJ, Lain S, Verma CS, Fersht AR, Lane DP. Awakening guardian angels: drugging the p53 pathway. Nat Rev Cancer 2009; 9:862-73.
2. Romer L, Klein C, Dehner A, Kessler H, Buchner J. p53—a natural cancer killer: structural insights and therapeutic concepts. Angew Chem Int Ed Engl 2006; 45:6440-60.
3. Bottger A, Bottger V, Sparks A, Liu WL, Howard SF, Lane DP. Design of a synthetic Mdm2-binding mini protein that activates the p53 response in vivo. Curr Biol 1997; 7:860-9.
4. Dastidar SG, Lane DP, Verma CS. Multiple peptide conformations give rise to similar binding affinities: molecular simulations of p53-MDM2. J Am Chem Soc 2008; 130:13514-5.
5. Kussie PH, Gorina S, Marechal V, Elenbaas B, Moreau J, Levine AJ, et al. Structure of the MDM2 oncoprotein bound to the p53 tumor suppressor transactivation domain. Science 1996; 274:948-53.
6. Madhumalar A, Lee HJ, Brown CJ, Lane D, Verma C. Design of a novel MDM2 binding peptide based on the p53 family. Cell Cycle 2009; 8:2828-36.
7. Zhong H, Carlson HA. Computational studies and peptidomimetic design for the human p53-MDM2 complex. Proteins 2005; 58:222-34.
8. McCoy MA, Gesell JJ, Senior MM, Wyss DF. Flexible lid to the p53-binding domain of human Mdm2: implications for p53 regulation. Proc Natl Acad Sci USA 2003; 100:1645-8.
9. Mayo LD, Turchi JJ, Berberich SJ. Mdm-2 phosphorylation by DNA-dependent protein kinase prevents interaction with p53. Cancer Res 1997; 57:5013-6.
10. Uhrinova S, Uhrin D, Powers H, Watt K, Zheleva D, Fischer P, et al. Structure of free MDM2 N-terminal domain reveals conformational adjustments that accompany p53-binding. J Mol Biol 2005; 350:587-98.
11. Showalter SA, Bruschweiler-Li L, Johnson E, Zhang F, Bruschweiler R. Quantitative lid dynamics of MDM2 reveals differential ligand binding modes of the p53-binding cleft. J Am Chem Soc 2008; 130:6472-8.
12. Worrall EG, Wawrzynow B, Worrall L, Walkinshaw M, Ball KL, Hupp TR. Regulation of the E3 ubiquitin ligase activity of MDM2 by an N-terminal pseudo-substrate motif. J Chem Biol 2009; 2:113-29.
13. Worrall EG, Worrall L, Blackburn E, Walkinshaw M, Hupp TR. The effects of phosphomimetic lid mutation on the thermostability of the N-terminal domain of MDM2. J Mol Biol 2010; 398:414-28.
14. Schon O, Friedler A, Freund S, Fersht AR. Binding of p53-derived ligands to MDM2 induces a variety of long range conformational changes. J Mol Biol 2004; 336:197-202.
15. Groban ES, Narayanan A, Jacobson MP. Conformational changes in protein loops and helices induced by post-translational phosphorylation. PLoS Comput Biol 2006; 2:32.
16. Ng YW, Raghunathan D, Chan PM, Baskaran Y, Smith DJ, Lee CH, et al. Why an A-loop phospho-mimetic fails to activate PAK1: understanding an inaccessible kinase state by molecular dynamics simulations. Structure 2010; 18:879-90.

17. Vogelstein B, Lane D, Levine AJ. Surfing the p53 network. Nature 2000; 408:307-10.
18. Vassilev LT, Vu BT, Graves B, Carvajal D, Podlaski F, Filipovic Z, et al. In vivo activation of the p53 pathway by small-molecule antagonists of MDM2. Science 2004; 303:844-8.
19. Dastidar SG, Lane DP, Verma CS. Modulation of p53 binding to MDM2: computational studies reveal important roles of Tyr100. BMC Bioinformatics 2009; 10:6.
20. Dastidar SG, Madhumalar A, Fuentes G, Lane DP, Verma CS. Forces mediating protein-protein interactions: a computational study of p53 "approaching" MDM2. Theor Chem Acc 2010; 125:621-635.
21. Brown CJ, Srinivasan D, Jun LH, Coomber D, Verma CS, Lane DP. The electrostatic surface of MDM2 modulates the specificity of its interaction with phosphorylated and unphosphorylated p53 peptides. Cell Cycle 2008; 7:608-10.
22. Lee HJ, Srinivasan D, Coomber D, Lane DP, Verma CS. Modulation of the p53-MDM2 interaction by phosphorylation of Thr18: a computational study. Cell Cycle 2007; 6:2604-11.
23. Brooks BR, Brooks CL, 3rd, Mackerell AD Jr, Nilsson L, Petrella RJ, Roux B, et al. CHARMM: the biomolecular simulation program. J Comput Chem 2009; 30:1545-614.